

U.S.S.N. 09/235,875

Filed: January 22, 1999

## AMENDMENT AND RESPONSE TO OFFICE ACTION

## In the Claims

1. (currently amended) A method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate in ~~E. coli~~ comprising providing bacteria expressing a phbA thiolase gene encoding an enzyme that converts butyryl-CoA and acetyl CoA to beta-ketohexanoyl-CoA, a phbB reductase gene that encodes an enzyme that converts beta-ketohexanoyl-CoA to beta-hydroxyhexanoyl-CoA, and a phbC polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl-CoA, the improvement ~~comprising expressing in the E. coli a D-specific enoyl-CoA hydratase and  $\beta$ -hydroxyacyl ACP-coenzyme A transferase, and providing feedstocks for the transgenic E. coli~~, wherein the enzymes are expressed in a sufficient amount to produce polyhydroxybutyrate-co-polyhydroxyhexanoate.

Claims 2-5 (Canceled).

6. (currently amended) The method of claim 1 wherein the phbC polymerase gene ~~encoding a PHA polymerase enzyme that incorporates C6 substrates~~ is incorporated into the bacterial chromosome.

7. (previously presented) The method of claim 1 wherein the phbC polymerase gene is from a bacteria selected from the group consisting of *Aeromonas caviae*, *Comamonas testosteroni*, *Thiocapsia pferigii*, *Chromatium vinosum*, *Bacillus cereus*, *Nocardia carolina*, *Nocardia salmonicolor*, *Rhodococcus ruber*, *Rhodococcus rhodocrous*, and *Rhodospirillum rubrum*.

Claims 8 and 9 (Canceled).

U.S.S.N. 09/235,875  
Filed: January 22, 1999  
AMENDMENT AND RESPONSE TO OFFICE ACTION

10. (currently amended) The method of claim 1 wherein the bacteria further comprises wherein the genes a gene encoding the D-specific enoyl CoA hydratase and  $\beta$ -hydroxyacyl-ACP-coenzyme A transferase are isolated from a bacterium selected from the group consisting of *R. eutropha*, *Klebsiella aerogenes*, *P. putida*, and *Aeromonas caviae*.

Claims 11-13 (Canceled).

14. (currently amended) The method of claim 1 ~~11~~ wherein the bacteria is *E. coli* expresses a ~~broad range reductase that is active on C6 substrates.~~

15. (currently amended) The method of claim 1 ~~11~~ wherein the ~~*E. coli*~~ expresses a gene encoding the polymerase that accepts 3-hydroxyhexanoyl CoA and 3-hydroxybutyryl CoA is from a bacterium selected from the group consisting of *R. eutropha*, *Klebsiella aerogenes*, *P. putida*, and *Aeromonas caviae*.

16. (currently amended) The method of claim 1 ~~11~~ wherein the ~~*E. coli*~~ bacteria expresses a ~~thiolase accepting acetoacetyl CoA~~ gene encoding a D-specific enoyl-CoA hydratase.

17. (currently amended) The method of claim 1 ~~11~~ wherein the bacteria *E. coli* expresses an ~~enzyme selected from the group consisting of thiolases specific for 3-ketohexanoyl CoA, reductase active on 3-ketohexanoyl CoA, and 3-hydroxyhexanoyl CoA~~ a PHB biosynthetic thiolase, the three enzymes from *C. acetobutylicum* that form butyryl CoA, the thiolase specific for 3-ketohexanoyl CoA, reductase specific for 3-ketohexanoyl, and PHB polymerase that accepts both 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA.

U.S.S.N. 09/235,875

Filed: January 22, 1999

**AMENDMENT AND RESPONSE TO OFFICE ACTION**

18. (currently amended) The method of claim 8 1 wherein the ~~*E. coli*~~ bacteria expresses one or more fatty acid biosynthetic enzymes.

19. (previously presented) The method of claim 18 wherein the fatty acid biosynthetic enzymes convert acyl ACP to acyl CoA.

20. (original) The method of claim 19 where the enzymes are selected from the group consisting of ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase.

21. (original) The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.

Claims 22- 34 (Canceled).